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Sera from patients with tuberculosis recognize the M2a-epitope (E2-subunit of pyruvate dehydrogenase) specific for primary biliary cirrhosis

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SUMMARY

Anti-M2 antibodies in primary biliary cirrhosis (PBC) have been shown to react with the alpha-ketoacid dehydrogenase complex of the inner mitochondrial membrane consisting of six epitopes (E2 subunit of the pyruvate dehydrogenase complex (PDC), 70 kD; protein X of the PDC, 56 kD; alpha-ketoglutarate dehydrogenase complex, 52 kD; branched-chain alpha-ketoacid dehydrogenase, 52 kD; E1alpha subunit of PDC, 45 kD; and E1beta-subunit of PDC, 36 kD). These epitopes are also present in the M2 fraction which is a chloroform extract from beef heart mitochondria. The E2 subunit of the PDC at 70 kD (M2a), especially, is a major target epitope which is recognized by about 85% of all PBC sera. However, analysing sera from 28 patients with active pulmonary tuberculosis it became evident that 12 (43%) also recognized the PDC-E2 subunit (M2a), as shown by Western blotting using the M2 fraction, the purified PDC, and the recombinant PDC-E2. In contrast, only two of 82 patients with other bacterial and viral infections including 25 patients with *Escherichia coli* infections reacted with the PBC-specific epitope at 70 kD. Naturally occurring mitochondrial antibodies (NOMA) were present in 54% of the patients with tuberculosis and in 50% of patients with other infectious disorders. They recognized either a determinant at 65 kD (epsilon) or at 60/55 kD (zeta/eta).None of the sera from 100 blood donors had anti-M2 but 14 had NOMA. Testing anti-M2 and NOMA-positive marker sera by Western blotting against membrane fractions derived from mycobacteria and *E. coli* it could be shown that—like mammalian mitochondria—they contain both the PBC-specific M2 antigen as well as the non-PBC-specific naturally occurring mitochondrial antigen system (NOMAg). The observation that PBC-specific antibodies were preferentially induced in patients suffering from a mycobacterial infection may provide some new clues to the still unknown etiology of PBC.

Keywords pyruvate dehydrogenase anti-M2 primary biliary cirrhosis tuberculosis mycobacteria

INTRODUCTION

In the last 5 years there was a great step forward in the characterization of the mitochondrial autoantigen M2, specific for the diagnosis of primary biliary cirrhosis (PBC). This antigen is known to be composed of five determinants at molecular weights 70 kD, 56 kD, 52 kD, 45 kD, 36 kD [1] corresponding to the E2-subunit of the pyruvate dehydrogenase complex (PDC-E2 = M2a), the protein X of PDC (M2b), the alpha-ketoglutarate dehydrogenase complex and the branched chain alpha-ketoacid dehydrogenase (M2c), the E1alpha-subunit of PDC (M2d), and the E1beta-subunit of PDC (M2e) [2–7]. The molecular characterization of the M2-antigen stimulated research in recent years, especially with respect to a better understanding of the etiopathogenesis of PBC.

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For the serological diagnosis of PBC, we have in the last 10 years used mainly the ATPase fraction prepared from beef heart mitochondria (named M2) in fluorometric immunoassay, ELISA, and Western blot [1,8,9]. Analysing more than 3000 PBC sera, we found a specificity of 99% and a sensitivity of 95% for the detection of anti-M2. However, we recently showed that this M2 fraction still contains another antigen system which is recognized by naturally occurring mitochondrial antibodies (NOMA) [10]. It consists of 12 epitopes with four major polypeptides at molecular weights 98 kD (alpha; present only in the M9 fraction) and 65 kD (epsilon; present only in the M2 fraction), and 60/55 kD (zeta/eta; present in both the M2 and M9 fraction). These epitopes are recognized preferentially by sera from patients with acute or chronic infectious disorders. Interestingly, some of these patients with infections, especially those with tuberculosis, revealed no NOMA but antibodies against the major PBC-specific determinant at 70 kD (M2a) [11,12]. We therefore extended these experiments by testing sera

Table 1. Definition of antigens present in different mitochondrial and bacterial preparations by anti-M2- and naturally occurring mitochondrial antibody (NOMA)-specific marker sera

	Marker sera*			
	Anti-M2 sera		NOMA sera	
Antigen preparation	Serum 1†, kD	Serum 2†, kD	Serum 3/4†, kD	Serum 5†, kD
Purified PDC‡	70, 56, 42	70, 56	—	60/55
Recombinant PDC-E2§	45	45	—	60/55
Mycobacterial membranes	72	72	72	55
Escherichia coli membranes	75/70	75/70	75/70	58/55
	52	52	62	

* Defined by their reaction pattern with the ATPase fraction M2 (M2a, 70 kD; M2c, 52 kD; M2d, 42 kD; M2e, 36 kD; NOMAg-epsilon, 65 kD; NOMAg zeta/eta, 60/55 kD).

† Serum 1, anti-M2a, c, d, e-positive; serum 2, anti-M2a-positive; serum 3/4, anti-epsilon-positive; serum 5, anti-zeta/eta-positive.

‡ Kindly provided by Dr S. Yeaman (UK).

§ Kindly provided by Dr M. E. Gershwin (USA).

PDC, Pyruvate dehydrogenase complex.

from patients with active tuberculosis and other infectious diseases including *Escherichia coli* infections (previously also assumed to produce PBC-specific antibodies [13–15]) for the presence of NOMA and PBC-specific humoral reactions, in order to clarify the relationship between the the PBC-specific 70 kD and the naturally occurring mitochondrial antigen (NOMAg)-specific epitope epsilon (65 kD) and to gain more insight into the etiology of PBC.

PATIENTS AND METHODS

Patients

Sera from 110 patients with different infectious diseases were examined: acute Epstein–Barr virus (EBV) infection ($n=25$), acute cytomegalovirus (CMV) infection ($n=32$), *E. coli* infections ($n=25$; five patients had an *E. coli* sepsis, 20 patients urinary tract infections), tuberculosis ($n=28$). The latter (25 males, three females; mean age 46·9 years, range 16–78 years) suffered from complicated pulmonary tuberculosis which showed cavitation in 11 patients. Diagnosis had been proven by cultural identification of *Mycobacterium tuberculosis*. Four patients were chronic alcohol abusers. All patients had received tuberculostatic therapy for 5–220 days. In most instances isoniazid and rifampicin had been applied. Seven patients showed an elevation of alkaline phosphatase, eleven of gamma-GT, five of ALAT, and five of ASAT (see also Table 3). Only two patients had increased IgM. Liver biopsy had not been performed.

Sera from 100 healthy blood donors served as negative controls.

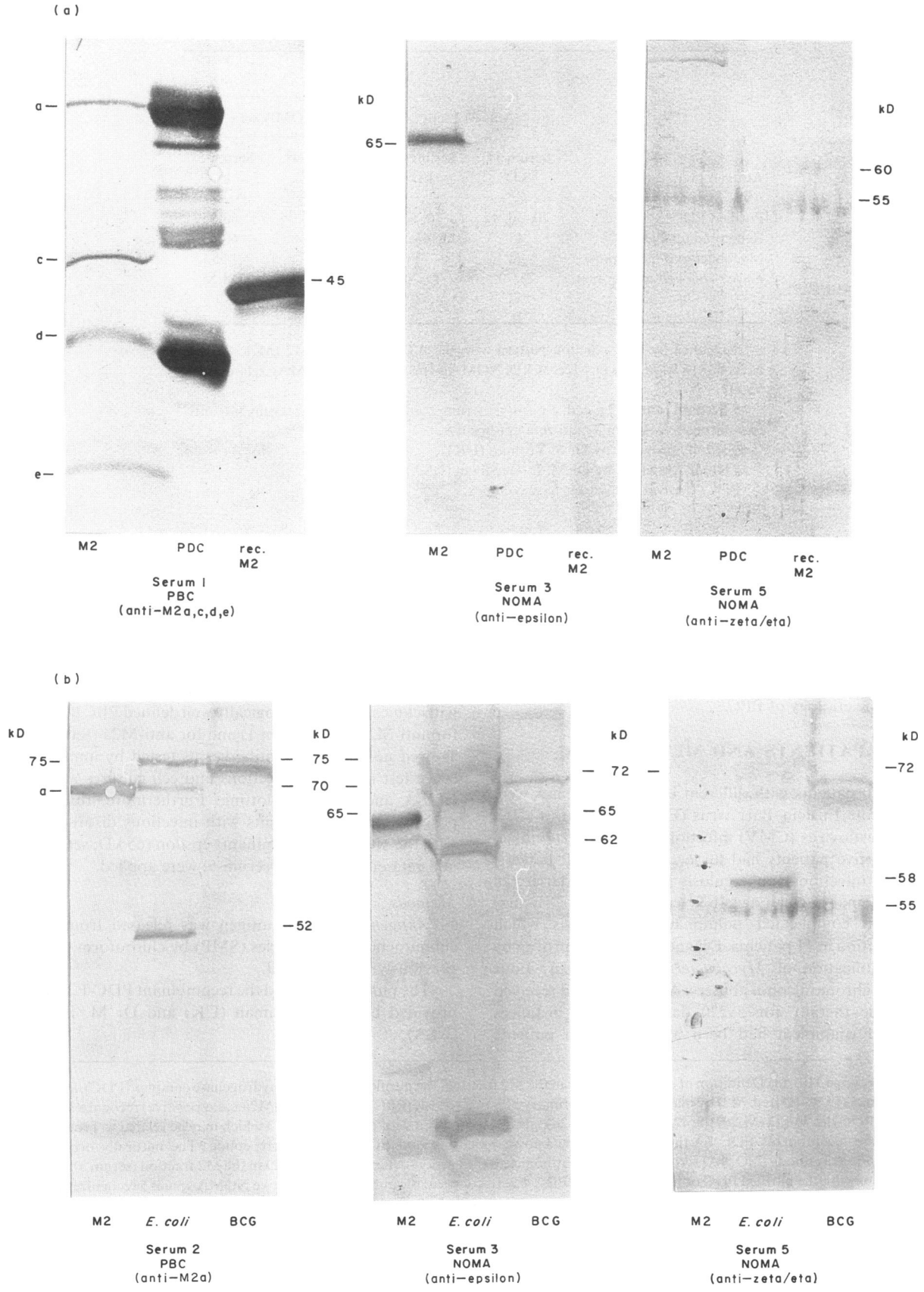
As positive controls we used two standard sera from patients with clinically and histologically well defined PBC being positive for anti-M2a,c,d,e (serum 1) and for anti-M2a (serum 2). They showed no other autoantibodies as tested by immunofluorescence test on cryostat sections and cell cultures as well as by ELISA and Western blotting. Furthermore, three NOMA-positive sera from patients with infectious disorders reacting with the NOMAg-determinants epsilon (65 kD; serum 3 and 4) and zeta/eta (60/55 kD; serum 5) were applied.

Methods

Antigens. The M2 antigen was released from beef heart submitochondrial particles (SMP) by chloroform treatment as previously described [16].

The purified PDC and the recombinant PDC-E2 were kindly provided by Dr S. Yeaman (UK) and Dr M. E. Gershwin (USA).

Fig. 1. (see page 310). (a) Definition of antigens present in the M2 fraction, the purified pyruvate dehydrogenase complex (PDC), and the recombinant PDC-E2 (rec. M2) by marker sera. The primary biliary cirrhosis (PBC) serum 1 (anti-M2a,c,d,e-positive) recognizes in the purified PDC the 70-kD (M2a), the 42-kD protein (M2d), and additionally a determinant at 56 kD which may be related to protein X, and in the recombinant PDC-E2 the 45-kD epitope known to correspond to the 70-kD polypeptide. The naturally occurring mitochondrial antibody (NOMA) serum containing antibodies to the epsilon-determinant at 65 kD in the M2 fraction (serum 3) shows no reaction with the purified PDC or the recombinant PDC-E2. In contrast, the anti-zeta/eta-positive NOMA serum 5 recognizes these epitopes also in the purified and recombinant PDC fractions and shows the typical fluffy band. (b) Reactivity of anti-M2- and NOMA-positive marker sera with membranes derived from *Escherichia coli* and mycobacteria bacille Calmette–Guérin (BCG). The PBC serum recognizing the 70-kD epitope (M2a) in the M2 fraction reacts with epitopes at 75, 70, and 52 kD on *E. coli* and at 72 kD on BCG. The same determinants are recognized by the anti-epsilon-specific NOMA serum on *E. coli* and BCG, and an additional determinant at 62 kD on *E. coli*. The anti-zeta/eta-specific serum reveals the eta-polypeptide at 55 kD with *E. coli* and BCG, and additional bands at 58 and 72 kD with *E. coli* and BCG respectively.


(a)
a—
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M2
PDC
rec. M2
Serum 1
PBC
(anti-M2a,c,d,e)
kD
65—
M2
PDC
rec. M2
Serum 3
NOMA
(anti—epsilon)
kD
—60
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M2
PDC
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Serum 5
NOMA
(anti—zeta/eta)
(b)
kD
75—
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M2
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Serum 2
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(anti—M2a)
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— 75 —
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65—
M2
E. coli
BCG
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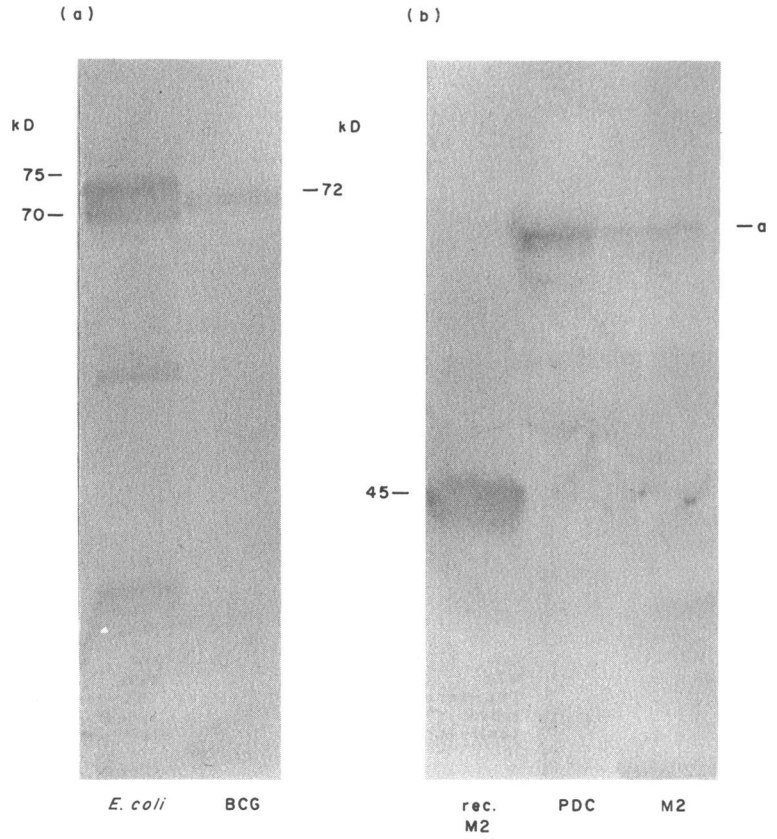

Fig. 2. Cross-reactivity between M2a (70 kD), the 75-kD and 70-kD bands of *Escherichia coli* membranes, and the 72-kD determinant of bacille Calmette–Guérin (BCG). (a) Antibodies were eluted from the M2a epitope after immunoblotting and retested against *E. coli* membranes and BCG. (b) Antibodies were also eluted from the 75-kD band from *E. coli* and retested against the recombinant pyruvate dehydrogenase complex (PDC) (rec.M2), the purified PDC, and the M2 fraction. Antibodies eluted from the 72-kD determinant of BCG gave the same reaction pattern as seen in (b).

Patients' sera were also tested against *E. coli* membranes prepared as recently described [1] as well as against mycobacterial membranes using bacille Calmette–Guérin (BCG; Medac, Hamburg). Both membrane fractions were sonified for 60 s before use.

Western blot. SDS–PAGE was performed with a 10% running gel and a 4·5% stacking gel based on the method of Laemmli [17].

Eighty micrograms of the M2-fraction, 1 μg of the cloned PDC-E2 and the purified PDC, 50 μg of *E. coli* membranes, and 10 μg of BCG were applied to the gels. The following immunoblots were carried out according to Towbin *et al.* [18] using immobilon membranes (Millipore, Bethesda, MD). Patients' sera were used at a dilution of 1:50. A serial dilution up to 1:2000 was performed with sera showing a positive reaction.

Elution studies. After immunoblotting, bound antibodies were eluted from their specific determinant by cutting out the bands of the immobilon sheets and dipping them into 0·2 M glycin buffer containing 0·5 M NaCl (pH 2·8). After 10 min, pH was neutralized, and the eluted antibodies were retested by Western blotting.

Immunofluorescence test. Patients' sera were tested by immunofluorescence (IFL) for a spectrum of autoantibodies according to Berg *et al.* [19] using cryostat sections from rat liver, kidney, heart, stomach, and human thyroid. Patients' sera were diluted 1:10 and incubated with the cryostat sections for 30 min. Bound antibodies were visualized by polyclonal fluorescent anti-human antibodies (Dako, Hamburg, Germany).

RESULTS

Definition of the mitochondrial and bacterial membrane fractions used in the Western blot by marker sera

The five standard sera recognizing PBC- and NOMAg-specific determinants were tested by Western blotting against the M2 fraction, the purified PDC, the recombinant PDC-E2, as well as the membrane fractions from *E. coli* and mycobacteria.

Both anti-M2-positive sera reacted with the 70-kD epitope in the purified PDC (M2a) and with a 45-kD protein in the human recombinant PDC-E2-fraction known to correspond to the 70-kD polypeptide (Table 1). The PBC serum positive for M2a,c,d,e (serum 1) recognized additionally M2d at 40 kD when tested against the purified PDC, as well as an epitope at 56 kD probably related to protein X (M2b) (Fig. 1a).

Analysing these fractions with NOMA-containing marker sera, it became evident that the purified PDC and the recombinant PDC-E2 did not contain the epsilon determinant at 65 kD but were still slightly contaminated with the zeta/eta polypeptides (60/55 kD; Table 1, Fig. 1a).

Testing the membrane fractions derived from *E. coli* and

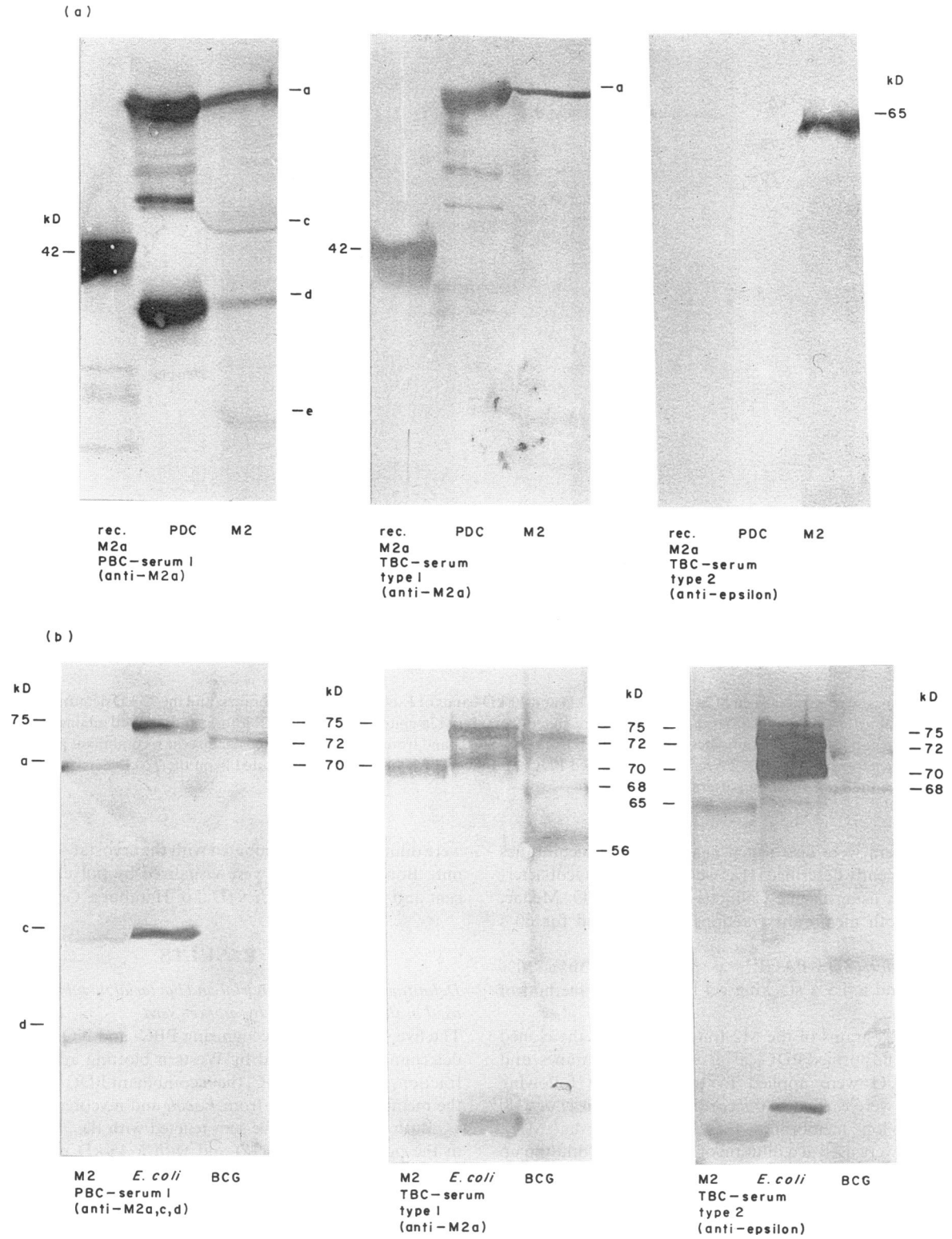

Fig. 3. (a) Demonstration of anti-M2a- and anti-epsilon-specific antibodies in sera from patients with tuberculosis. The TBC-serum 1 (anti-M2a-positive) reacts with the 70-kD determinant (M2a) when tested against the M2 fraction, the purified pyruvate dehydrogenase complex (PDC), and the cloned PDC (rec. M2). In contrast, the TBC-serum type 2 (anti-epsilon-positive) reacts with the NOMAg determinant epsilon (65 kD) only in the M2 fraction, but is negative with the purified and recombinant PDC. (b) Reactivity of the TBC-serum type 1 (anti-M2a-positive) and type 2 (anti-epsilon-positive) with membranes derived from *Escherichia coli* and mycobacteria bacille Calmette–Guérin (BCG). Like the anti-M2-positive primary biliary cirrhosis (PBC) serum, the TBC-serum type 1 recognizes on *E. coli* the 74-kD and 70-kD and on BCG the 72-kD determinants. With BCG it shows two additional epitopes at 68 and 56 kD. The TBC-serum type 2 also reacts with the 75/70-kD and the 72-kD and 68-kD polypeptides on *E. coli* and BCG, respectively.

Table 2. Recognition of primary biliary cirrhosis (PBC)- and NOMAg-specific epitopes in the Western blot by sera from patients with different infectious diseases at a serum dilution of 1:50

Groups	Number of patients	PBC-specific* M2a, 70 kD	NOMAg-specific† Epsilon, 65 kD, % positive	NOMAg-specific† Zeta/eta, 60/55 kD
Patients with				
Tuberculosis	28	43‡	54	32
Escherichia coli infection	25	0	52	12
EBV-infection	25	4	32	56
CMV infection	32	3	17	34
Blood donors	100	0	10	4

* Using the M2 fraction, the purified pyruvate dehydrogenase complex (PDC) and the recombinant M2a antigen.

† Using the M2-fraction.

‡ Additionally, 16% reacted with the PBC-specific M2c determinant at 52 kD.

EBV, Epstein–Barr virus; CMV, cytomegalovirus.

mycobacteria against the M2- and NOMA-specific marker sera, it could be shown that both the M2a- and the NOMAg-epitopes were present in both preparations (Fig. 1b). Thus, anti-M2 as well as anti-epsilon (65 kD) recognized a 75-kD and a 70-kD determinant on *E. coli*, and a 72-kD band on mycobacteria. In contrast, the anti-zeta/eta positive NOMA serum showed the previously described 55-kD determinant (eta) in all three fractions and reacted with an additional protein at 58 kD on *E. coli* membranes.

In order to see whether the 75-kD and 70-kD polypeptides on *E. coli*, and the 72-kD epitope on BCG correspond to the E2 subunit of PDC (M2a), antibodies were eluted from these bands after immunoblotting, and retested against all antigen fractions including the purified PDC and the recombinant PDC-E2. Antibodies eluted from the 70-kD and 75-kD determinants of *E. coli* membranes as well as those eluted from the 72-kD polypeptide of BCG reacted with M2a in the M2 fraction, the purified PDC, and the cloned PDC-E2. Furthermore, antibodies eluted from the M2a determinant recognized the 70-kD and 75-kD epitopes on *E. coli* and the 72-kD band on BCG, indicating that these polypeptides and the M2a epitope are identical (Fig. 2).

Furthermore, we also tried to elute NOMA reacting with the determinant epsilon at 65 kD in the M2 fraction, the 75-kD and 70-kD epitopes on *E. coli* and the 72-kD polypeptide on mycobacteria. However, compared with the eluted anti-M2a antibodies, no specific reaction was obtained after retesting these NOMA in the Western blot against the different antigen fractions.

Recognition of the E2 subunit of PDC (M2a) by sera from patients with tuberculosis

Based on the observation that bacterial membranes express M2 epitopes, we tested sera from patients with tuberculosis and *E. coli* infections as well as other infectious disorders for antibodies against M2. Table 2 shows that 43% of 28 patients with pulmonary tuberculosis recognized the M2a epitope in all three antigen fractions (M2, PDC, recombinant PDC-E2) as demonstrated by Western blotting. This reaction was observed up to a serum dilution of 1:500 in some instances. Fifty-four percent of the patients with tuberculosis had antibodies against the NOMAg-determinant epsilon. Only three patients expressed neither anti-M2 nor NOMA. There was no correlation between antibody pattern and kind or duration of tuberculostatic therapy. Furthermore, sera from two patients with EBV and CMV infection reacted with PDC-E2.

Interestingly, no M2-specific antibody activity could be detected in sera from patients with *E. coli* infections, although anti-epsilon antibodies were found in 52% of the 25 patients.

Figure 3 shows the typical M2a- and epsilon-specific reaction using either an anti-M2a-positive/anti-epsilon-negative serum (type 1) and an anti-M2-negative/anti-epsilon-positive serum (type 2) from two patients with tuberculosis. The sera were tested either against the M2 fraction, the purified PDC, and the recombinant PDC-E2 (Fig. 3a) or against *E. coli* and BCG-membrane fractions (Fig. 3b). The reaction patterns were basically the same as determined with anti-M2a- and NOMA-specific marker sera.

Other autoantibodies in patients with tuberculosis

Screening the 28 sera from patients with tuberculosis for other autoantibodies by IFL on cryostat sections, 14 (50%) were positive. Seven had antibodies against parietal cells of the stomach, two had antibodies to smooth muscle antigens, two against endothelial cells, two against sarcolemma, and one against nuclei. None of them revealed the typical antimitochondrial antibody pattern.

Biochemical parameters in patients with tuberculosis

The biochemical parameters of the tuberculosis patients in relation to the mitochondrial antibody patterns are given in Table 3. Patients being anti-M2a-positive, i.e. recognizing the PBC-specific PDC-E2 subunit, more frequently had pathological levels of alkaline phosphatase and gamma-GT compared with NOMA-positive patients; however, mean values did not differ significantly.

Only two patients showed an elevation of serum IgM and four of increased IgA levels. Two of them suffered from an alcoholic liver disease.

Table 3. Biochemical parameters in 28 patients with acute tuberculosis in relation to the presence of different mitochondrial antibody types*

Biochemical parameters	Patients with tuberculosis		
	Anti-M2a-positive† ($n=12$)	NOMA-positive‡ ($n=13$)	Anti-M2 and NOMA-negative ($n=3$)
AP (>200 U) number (%)	4 (33)	2 (15)	1 (33)
mean	169·1	161·3	218·7
range	104–232	89–330	114–354
Gamma-GT (>28 U) number (%)	6 (50)	3 (23)	2 (66)
mean	34·8	26·4	32·3
range	9–131	3–71	24–41
ALAT (>20 U) number (%)	2 (17)	2 (15)	1 (33)
mean	12·8	17·6	11·3
range	3–42	6–66	6–20
ASAT (>20 U) number (%)	1 (8)	4 (31)	0
mean	8·5	16·2	9·3
range	1–30	2–36	5–14
IgM (>285 mg%) number (%)	1 (8)	1 (8)	0
mean	156·3	124·8	93·3
range	62–367	25–304	93–94
IgA (>485 mg%) number (%)	0	3 (23)§	1 (33)
mean	285·0	416·9	377·3
range	63–381	190–565	218–492

* Values are given before tuberculostatic therapy.
† Five patients also had naturally occurring mitochondrial antibody (NOMA).
‡ Antibodies to the determinants epsilon (65 kD) and zeta/eta (60/55 kD).
§ Two patients suffered from alcoholic liver disease.

DISCUSSION

In this study it is shown that sera from patients with tuberculosis, in particular, can recognize in the Western blot the PBC-specific M2a epitope, i.e. the E2 subunit of PDC. Thus, 12 (43%) of 28 tuberculosis patients had antibodies against this determinant at 70 kD using the M2 fraction derived from beef heart mitochondria, the purified PDC, and the recombinant PDC-E2. Although four of these patients showed slightly elevated alkaline phosphatase levels, and six pathological gamma-GT, there was otherwise clinically little evidence that these patients suffered from PBC. Only one patient had elevated IgM, and only four of the 28 patients were females.

Anti-M2a titres in patients with tuberculosis were rather low, ranging from 1:50 to 1:500 when tested by Western blotting using the ATPase associated antigen fraction. However, a 'non-specific' effect due to the low serum dilution can be excluded with high probability in view of our long-lasting experience applying this method for the diagnosis of PBC, hereby also using serum dilutions of 1:50.

We cannot explain yet why these anti-M2-positive tuberculosis sera gave no mitochondrial antibody pattern in the immunofluorescence test. However, this phenomenon could also be observed in some patients with clinically and histologically well defined PBC showing high anti-M2 antibody titres in ELISA and Western blotting, but being antimitochondrial antibody (AMA)-negative in the IFL. On the other hand, there are also PBC sera with high AMA titres in the IFL which did not react with M2 epitopes in ELISA and Western blot. These findings indicate that IFL and ELISA or Western blot may—at least in some instances—detect different antibody specificities.

From the observation that patients with tuberculosis can produce PBC-specific antibodies, the question arises whether mycobacterial membranes can induce the production of specific antimitochondrial antibodies by their ability to express M2 epitopes on their membranes. Analysing *E. coli* and mycobacterial membranes by PBC- and NOMA-specific marker sera, it became evident that both antigen fractions contain the PBC-specific M2a (70 kD) as well as the NOMAg-specific epsilon (65 kD) and zeta/eta-epitopes (60/55 kD).

Although the epsilon band and the PDC-E2 had almost the same molecular weight, they could be clearly distinguished, since only the PBC-specific anti-M2a antibodies reacted with the purified PDC and the recombinant PDC-E2 in parallel, while anti-epsilon antibodies reacted only with the ATPase fraction but not with the purified or cloned PDC-E2. It should be mentioned, however, that the NOMA with anti-zeta/eta specificity (60/55 kD) recognized their epitopes in the purified as well as the cloned PDC-E2 fraction, indicating also some contamination with NOMAg as demonstrated for the ATPase-(M2) fraction.

The presence of M2a on *E. coli* and mycobacteria was further proven by eluting antibodies from M2a after immunoblotting and retesting them against the bacterial membranes

(and *vice versa*). These findings are in contrast to Fussey *et al.* [20], who concluded from their experiments that PDC (M2a) on bovine heart mitochondria is different from that on *E. coli*, and that PBC sera may contain two different antibody types against mammalian and prokaryotic PDC. The fact that the PDC-E2 and the epsilon-NOMAg epitopes have very similar molecular weights would explain the findings of some authors that sera from patients with urinary tract infections react with the 'PBC-specific 70-kD determinant' [13,14]. It seems, however, more likely that this reactivity was due to the presence of antibodies to the NOMAg epitope epsilon, considering also our finding that none of our patients with *E. coli* infections had antibodies against PDC-E2 (M2a) but 52% against epsilon. Stemerowicz *et al.* immunized rabbits with rough mutants of enterobacteriaceae and found antibodies against the 70-kD epitope testing the sera by Western blotting against beef heart mitochondria [15]. However, since this fraction contains both PBC- and NOMAg-specific epitopes, it still has to be proven whether this reaction was caused by PBC- or NOMA-specific antibodies.

The mechanisms which trigger the production of anti-M2 antibodies in PBC remain unclear. An infectious agent has not been found in the liver or blood of PBC patients. Removal of the diseased liver is without influence on the formation of anti-M2 antibodies [21], again indicating that the anti-M2-specific B cell clone seems not to need any liver-specific antigen stimulation. The similarities in molecular weights of the NOMAg-specific epitope epsilon and the PBC-specific 70-kD epitope could indicate that the anti-M2 antibodies are also derived from the pool of naturally occurring mitochondrial antibodies. Our observation that the epsilon-determinant is a mitochondrial protein [10] would fit this hypothesis. Somatic point mutation or slight differences in cross-reacting epitopes would easily explain differences in the antigenic specificity of these antibodies.

It has been suggested recently that heat shock proteins (hsp) play a major role in autoimmune diseases [22], and hsp 65, a mitochondrial protein which is also expressed on mycobacterial membranes, has a molecular weight (65 kD) in the same range as M2a and epsilon. However, from preliminary experiments we know that anti-M2-positive PBC sera as well as NOMA-positive sera do not recognize hsp 65, and that antibodies to hsp 65 do not react with M2a or epsilon (unpublished observation; cloned mycobacterial hsp 65 as well as monoclonal and polyclonal antibodies to hsp 65 were kindly provided by Dr S. H. E. Kaufmann, Ulm, Germany). Nevertheless, cross-reactivity with another epitope derived from an infectious agent still seems a likely explanation for the stimulation of disease-specific anti-M2 antibodies. In this respect, *E. coli*, as previously suggested [15], seem not to be of major importance as a causative agent. Mycobacteria may be another candidate. Several 'autoimmune' or 'idiopathic' diseases, including Takayasu's arthritis, rheumatoid arthritis, Crohn's disease, ulcerative colitis, sacroileitis, and psoriasis, are considered to be triggered by slow bacterial infections, and especially in Takayasu's and rheumatoid arthritis there is a clear association with mycobacterial infections [23]. Furthermore, also in sarcoidosis mycobacteria were recently identified by polymerase chain reaction (PCR) [24]. Therefore, atypical mycobacterial infections may also be considered to play a role in the etiopathogenesis of PBC. By analogy with tuberculosis [25], one could speculate that in PBC macrophages are chronically infected, thereby continuously presenting some cross-reacting epitopes on their membranes, which are then responsible for the ongoing activation of a certain B cell clone.

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